Published in final edited form as:
Nutr Cancer. 2012 February ; 64(2): 307–314. doi:10.1080/01635581.2012.648819.

Urinary estrogen metabolites during a randomized soy trial

Yukiko Morimoto[1], **Shannon M. Conroy**[1], **Ian S. Pagano**[1], **Marissa Isaki**[1], **Adrian A. Franke**[1], **Frank J. Nordt**[2], and **Gertraud Maskarinec**[1]

[1]University of Hawaii Cancer Center, Honolulu, HI

[2]Rhein Consulting Laboratories, Portland, OR

Abstract

One of the hypothesized protective mechanisms of soy against breast cancer involves changes in estrogen metabolism to 2-hydroxy (OH) and 16α-OH estrogens. The current analysis examined the effect of soy foods on the 2:16α-OH E_1 ratio among premenopausal women during a randomized, crossover intervention study; women were stratified by equol producer status, a characteristic thought to enhance the protective effects of soy isoflavones. The study consisted of a high-soy diet with 2 soy food servings/day and a low-soy diet with <3 servings of soy/week for 6 months each; estrogen metabolites were measured in 3 overnight urines (baseline and at the end of the low- and high-soy diet) using gas chromatography mass spectrometry for the 82 women who completed the study. Urinary isoflavonoids were assessed by liquid chromatography mass spectrometry. When applying mixed models, the 2:16α-OH E_1 ratio increased (p=0.05) due to a non-significant decrease in 16α-OH E_1 (p=0.21) at the end of high-soy diet. Similar non-significant increases in the 2:16α-OH E_1 ratio were observed in equol producers (p=0.13) and non-producers (p=0.23). These findings suggest a beneficial influence of soy foods on estrogen metabolism regardless of equol producer status.

Keywords

Breast cancer risk; diet and nutrition; estrogen metabolites; isoflavones; soy foods

INTRODUCTION

Soy has been associated with protection against breast cancer, primarily because of low breast cancer rates in Asian countries where soy foods are regularly consumed (1). A number of biological mechanisms have been proposed for the protective effect of soy intake; one involves modification of estrogen synthesis and metabolism (2). Estrogen exposure is known to be a key factor in breast cancer development (3). Therefore, soy consumption is hypothesized to lead to a more favorable or cancer-protective pattern of estrogen metabolites (4,5). The metabolism of E_1 and E_2 follows 3 competing pathways, C2-, C4- and C16α-hydroxylation (6–8), of which 2-hydroxy (OH) estrogen metabolites appear to inhibit breast cell proliferation while 16α-metabolites have a more carcinogenic potential that promotes different steps of tumorigenesis (3,8). Thus, the ratio of 2-OH E_1 to 16α-OH E_1 may serve as a risk indicator of estrogen exposure associated with breast cancer risk. A lower 2:16α-OH E1 ratio was observed in breast cancer survivors in comparison to healthy controls (9). However, the current evidence linking the 2:16α-OH E_1 ratio with breast cancer is limited and inconsistent (10–14). A recent study among pre- and postmenopausal Asian American

Address for correspondence: Gertraud Maskarinec, MD, PhD, University of Hawaii Cancer Center, 1236 Lauhala Street, Honolulu, HI 96813, Phone: (808) 586-3078, FAX: (808) 586-2982, gertraud@cc.hawaii.edu.

women provided support that the urinary 2:16α-OH E_1 ratio differs according to breast cancer risk and that the 2:16α-OH E_1 ratio may be a marker for Western lifestyle influences on estrogen metabolism (15). This is a finding of interest given the increasing breast cancer rates in the subsequent generations of Asian migrants to the United States (16) and the stronger protective effect of soy foods in Asian than Western populations (1). In this study, we examined the effect of daily consumption of soy foods on urinary 2-OH E_1 and 16α-OH E_1 concentrations and on the 2:16α-OH E_1 ratio among non-lactating, premenopausal women of diverse ethnicities in Hawaii who participated in an intervention trial. Furthermore, we stratified the women by equol producer status, a characteristic thought to enhance protective effects of soy isoflavones.

MATERIALS AND METHODS

Study design and procedures

We conducted a randomized, crossover soy intervention study with two 6-month diet periods (high-soy and low-soy) separated by a 1-month washout period (17). Women were excluded if they consumed more than 5 soy servings per week, had breast implants, used estrogen-containing oral contraceptives, were pregnant or breast-feeding, had been diagnosed with cancer, or did not have a uterus or regular menstrual periods. At the initial screening visit, the women completed demographic and diet questionnaires, weight and height measurements, a 24-hour dietary recall, and a nipple aspirate fluid (NAF) collection. Of potential participants who produced at least 10 μL of NAF, 96 women were randomly assigned to begin with either the high-soy (Group A) or low-soy (Group B) diet and crossed over to the other diet after a one-month washout. Randomized participants completed a self-administered, 26-page food frequency questionnaire (FFQ) (18) and a special 1-page soy questionnaire that assessed dietary intake during the previous year. Subsequently, 14 women (15%) dropped out. After the randomization visit, participants attended 5 subsequent visits at midway (months 3 and 11) and at the end (months 6 and 13) of each diet period and after washout period (month 7). Body weight was measured at each study visit. Three unannounced, 24-hour diet recalls were completed in each diet period at months 1, 3 and 5, and 8, 10, and 12. The recalls, as well as the baseline FFQs, were analyzed for daily intake of soy and other foods and nutrients (17). The mean intake of 3 diet recalls was calculated for each diet period. The 82 women were classified into three ethnic categories: Caucasian (42), Asian (3 Chinese, 5 Filipino, 12 Japanese, and 2 Korean), and Other (1 American Indian, 14 Native Hawaiian, and 3 Others).

The goal during the high-soy diet period was to consume 2 daily servings of soy foods, 7 days per week; one serving was defined as ¾ cup of soy milk, ½ cup of tofu or ¼ cup of soy nuts and provided approximately 25 mg of isoflavone aglycone equivalents per serving. A 3-month supply of soy foods was provided at the beginning and the middle of the high-soy diet. During the low-soy diet period, participants were instructed to maintain their usual diet and limit consuming soy-containing products to <3 servings per week. Dietary intake and adherence to the intervention strategy throughout the study were assessed by urinary isoflavonoid excretion and by 6 unannounced 24-hour dietary recalls (Table 2). The Committee on Human Subjects at the University of Hawaii and the participating clinics approved the study protocol. All participants signed an informed consent form. A Data Safety Monitoring Committee annually reviewed study progress, reasons for dropouts, and any reported symptoms. The study was registered at clinicaltrials.gov as NCT00513916. Further details of the study methods and dietary compliance to the soy intervention were described previously (17).

Urine collection and analysis

The study participants provided 8 urine samples throughout the study; 6 overnight urine samples were brought to study visits (at baseline and months 3, 6, 7, 10, and 13), and 2 morning urine samples were sent by mail in months 1 and 8. All study visits were planned to occur during the mid-luteal phase (3–11 days before the next menstruation); however, due to scheduling problems, only 58% of actual visits took place during that period; 10% were closer to the next menstruation, 25% occurred during the follicular phase, and for 7% timing could not be determined. A mixture of boric and ascorbic acid was added to the air-tight plastic urine containers to lower the pH and to stabilize the isoflavonoids without interfering with the assay. Subsequently, the 3 overnight urine samples from baseline, month 6, and month 13 were analyzed for estrogens and their metabolites to evaluate the effect of the soy diet on the estrogen metabolite concentrations. One woman did not provide a baseline sample, 2 women each missed the month 6 and 13 collection; these samples were recorded as missing.

Because isoflavones are specific to soy foods and are excreted in urine within 24–36 hours of consumption, urinary isoflavonoid assessment is an excellent biomarker for soy intake. Isoflavonoids in urine (daidzein, genistein, equol, and O-desmethylangolensin) were measured using liquid chromatography tandem mass spectrometry (LCMS) after enzymatic hydrolysis and liquid-liquid extraction as described previously (19). The equol producer status of each collection period (eight per participant) was determined based on the combination of two criteria: urinary daidzein excretion ≥2 nmol/mg and urinary equol to daidzein ratio ≥0.018. Participants that met both criteria for at least one of the eight urine collections were classified as equol producers (n=43); of these 43 women, 16 were positive once, 8 twice, 7 three times, 8 four times, 3 five times, and 1 six times. To adjust for urinary volume, the urinary creatinine levels were analyzed with a Roche-Cobas MiraPlus chemistry analyzer using a kit from Randox Laboratories (Crumlin, UK) that is based on a kinetic modification of the Jaffé reaction; isoflavone excretion was expressed as nmol/mg creatinine (20,21).

To measure urinary estrogen metabolites at baseline, the end of the low and high-soy diet, gas chromatography mass spectrometry (GCMS) analysis (one of the authors; FJN) was performed according to the methods described previously (22) using a model HP-6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) and a model Agilent 5973 single quadruple mass spectrometer. For quantitation of total steroids, representing the sum of free, sulfated and glucuronidated steroids, 10 ml of urine was extracted using C-18 (octadecylsilyl) solid phase extraction (SPE) columns (500 mg extraction bed, Varian, Walnut Creek, CA) and eluted with methanol. The eluate, after drying, was subjected to hydrolysis using a mixture of β-glucuronidase/aryl sulphatase (Helix pomatia, Roche Molecular Biochemicals, Indianapolis, IN) and crude β-glucuronidase (Helix pomatia, Sigma, St. Louis, MO) in sodium acetate buffer pH 4.8. This digest was again extracted with the C-18 SPE cartridge and the adsorbed steroids eluted with methanol, dried down and derivatized in a two- step process to the methyloxime-trimethylsilyl (MO-TMS) ethers. First the dried extract was incubated at 60°C for 1 hour in the presence of 2% (w/V) methoxyamine hydrochloride (Sigma) in pyridine. The pyridine was blown off and trimethylsilylimidazole (Regis Technologies, Morton Grove, IL) was added followed by incubation overnight at 100°C. Derivatization products were purified by taking them up in a small volume of cyclohexane and passing them through a Lipidex column (Hydroxyalkoxy-propyldextran, Type IX, Sigma). Finally, an aliquot (equal or less than 2 μL) of the concentrated eluate in cyclohexane was injected onto a temperature controlled non-polar capillary GC column (e.g., HP-1MS, 30 m, Agilent). Steroids were eluted essentially as described earlier (22), identified and quantified by single ion monitoring (SIM) with HP-Chemstation software (Agilent) for integration of peak areas. A mixture of E_1-d_4, E_2-d_4 5α-

androstan-3α, 17α-diol, stigmasterol and cholesteryl butyrate (Steraloids, Newport, RI) served as internal standards. E_1–d_4 served as the internal standard for E_1, E_2–d_4 served as internal standard for E_2 and E_3 whereas 5α-androstan-3α, 17α-diol and stigmasterol served as the internals standard for the other compounds. Blinded urine samples served as quality control samples and indicated both acceptable reproducibility and accuracy of the assay. Cholesteryl butyrate did not get derivatized and was used to monitor efficacy of the column. The following estrogens and metabolites were measured with the method described above: E_1, E_2, E_3, 2-OH E_1, and 16α-OH E_1. Then, the ratio of 2-OH E_1 to 16α-OH E_1 was calculated.

Statistical analysis

The statistical analysis was performed with the SAS statistical software package version 9.2. (SAS Institute, Inc., Cary, NC). To assess differences in baseline characteristics between the two randomization groups (Groups A and B), Student's *t* tests and χ^2 tests were performed for continuous variables (age at screening, BMI, age at menarche, number of children, age at first live birth, breast-feeding duration, urinary isoflavone excretion, and dietary isoflavone intake) and for categorical variables (ethnicity and equol producer status), respectively. Due to the non-normal distributions, urinary estrogen metabolite data were log-transformed prior to statistical analysis. Medians and interquartile ranges were plotted for all women and separately by equol producers and non-producers. Because of the repeated measures, mixed models were used to detect the effect of the high-soy diet in comparison to baseline and the low-soy diet; models also included a group and time (months 0, 6 and 13) variable as well as interaction terms. Subgroup analyses stratified the participants by equol producer status.

RESULTS

The two randomized groups were similar in baseline characteristics including ethnicity, BMI, age at menarche, number of children, age at first live birth and breast-feeding duration (Table 1). Differences were noted in the age at study entry and urinary isoflavone excretion; Group A was slightly older than Group B (41.3±5.6 vs. 37.3±6.0; $p<0.01$) and excreted more urinary isoflavones (728±1133 vs. 281±607 pg/mL; $p=0.03$). However, usual soy intake during the last year (5±8 and 7±10 g/day for Groups A and B, respectively; $p=0.44$) did not differ by group. Equol producer status and urinary estrogen metabolite excretion at baseline were also similar in both groups. In addition, equol producer status showed no difference across ethnic groups ($p=0.66$).

In all women, the high-soy diet was associated with modest changes in 2-OH E_1 and 16α-OH E_1 levels, whereas no significant differences were observed for E1, E2, and E3 (Table 2). The median (and interquartile range) for urinary 2-OH E_1 excretion was 5.0 (2.2–9.3) ng/mL at baseline, 4.6 (1.8–9.6) ng/mL at the end of the low-soy diet, and 5.1 (1.9–11.6) ng/mL at the end of the high-soy diet (Figure 1). The respective values for 16α-OH E_1 were 1.8 (0.3–2.8) ng/mL at baseline, 1.6 (0.5–3.1) ng/mL after low-soy, and 1.3 (0.3–2.8) ng/mL after high-soy. For the 2:16α-OH E_1 ratio, the median was 3.2 (1.6–8.0) at baseline, 2.8 (1.2–6.0) at the end of the low-soy diet, and 4.2 (2.2–7.3) at the end of the high-soy diet. In mixed models, the high-soy diet showed a non-significant decrease in 16α-OH E_1 ($p=0.21$) and a marginally significant increase in the 2:16α-OH E_1 ratio ($p=0.05$) in all women as compared to baseline and the low-soy diet, whereas no change in 2-OH E_1 ($p=0.40$) was detected. An interaction term of soy treatment with ethnicity was not statistically significant ($p=0.97$) for the 2:16α-OH E_1 ratio.

Of 82 women, 43 were classified as equol producers and 39 as non-producers. Similar increases in the 2:16α-OH E_1 ratio after high-soy diet were observed in separate analyses for equol producers ($p=0.13$) and non-producers ($p=0.23$). The median urinary 2:16α-OH E_1

ratio (and interquartile range) for equol producers was 3.6 (1.8–9.1) at baseline, 3.1 (1.3–6.3) at the end of the low-soy diet, and 4.3 (2.3–7.3) at the end of the high-soy diet, whereas the respective values for non-producers were 3.0 (1.0–6.1), 2.5 (1.2–4.3), and 4.2 (1.7–7.9). Also, the pattern of 16α-OH E_1 and 2-OH E_1 did not differ by equol producer status. Interaction terms between soy treatment and equol producer status were not significant ($p=0.15$ for 2-OH E_1; $p=0.52$ for 16α-OH E_1; and $p=0.66$ for the 2:16α-OH E_1 ratio). Inclusion of randomization group in the model did also not change the results, and an interaction term between time and diet was not significant ($p=0.53$). Interestingly, a significant intervention effect ($p=0.03$) was detected for E_3 for the equol producers only due to the relatively high E_3 value at the end of the low-soy period.

DISCUSSION

This study observed a possible influence of 2 daily soy food servings on urinary estrogen excretion of 2-OH E_1 and 16α-OH E_1, but not levels of E_1, E_2, and E_3 among non-lactating, premenopausal women of diverse ethnicities. An increase in the 2:16α-OH E_1 ratio at the end of a 6-month high-soy diet was clearly seen across all women, regardless of equol producer status, although the statistical significance was borderline. These findings provide support for a protective effect of soy against breast cancer via its potential ability to modify estrogen metabolism that results in a lower exposure to more carcinogenic metabolites.

The results of the present study agree with some of the previous reports (2,4,9,23) that described an increase in the 2:16α-OH E_1 ratio and disagree with others (24,25) that observed no effect on the 2:16α-OH E_1 ratio after a soy intervention. The studies varied in feeding duration but were generally short-term (ranging from 1–3 menstrual cycles to 100 days) and mostly used soy protein or highly processed isoflavone-containing products. Among the studies that described an increased 2:16α-OH E_1 ratio, more studies observed an increase in 2-OH E_1 excretion than a decrease in 16α-OH E_1 or changes in both 2-OH E_1 and 16α-OH E_1, whereas the current study observed a decrease in 16α-OH E_1. Interestingly, a large cross-sectional study that examined urinary estrogen excretion of Asian Americans by tertiles of adult soy food consumption observed a trend in both increasing 2-OH E_1 and decreasing 16α-OH E_1 across menopausal status (5). Inconsistent findings may be partly due to differences between soy foods and isoflavone supplements, menopausal status of the study populations, and greater fluctuation in estrogen levels among premenopausal women. The lack of a difference by equol producer status is in contrast to a recent trial among 40 postmenopausal women (9), in which soy protein supplementation increased the 2:16α-OH E_1 ratio ($p=0.04$) only among equol producers. Equol is metabolized from the isoflavone daidzein predominately by intestinal bacteria, and individuals who have the capacity to produce equol, approximately 30–50% of the population, are thought to experience enhanced protective effects of isoflavones when compared to non-producers (26,27). However, as far as we know, none of the other studies determined the equol producer status of the participants. The puzzling observation that women change equol producer status over time was also seen in both pre and postmenopausal women previously (28,29). It challenges the widely held belief that equol production remains stable and cannot be fully explained at this time.

This study has several strong points including its randomized, crossover design with a relatively long intervention period with repeated sampling and the diverse ethnic backgrounds of the participants. We also used soy foods that are consumed in Asian countries as opposed to soy protein or isoflavone supplements and administered amounts similar to typical intakes in Asia (30). A relatively large sample size allowed a comparison by equol producer status, which showed similar patterns of higher 2:16α-OH E_1 ratio in equol producers and non-producers at the end of high-soy diet period. The use of GCMS to

measure urinary estrogen metabolite excretion provided more accurate results than the commonly-used Enzyme-Linked Immunosorbent Assay (ELISA) method that has limited capacity to measure low estrogen levels; a recent comparison of the ELISA method to a newly developed liquid chromatography tandem mass spectrometry (LCMS) observed a poor ability of the former method in detecting estrogen excretions of premenopausal women (31).

This study had a number of limitations. One major issue is the observed differences in baseline characteristics of the two randomization groups, namely, age and urinary isoflavone excretion because the randomization did not achieve perfectly balanced groups, but the observed group differences in urinary isoflavone levels likely reflected varying soy intakes among the women on a single day rather than true group differences in usual soy intake. Combined analysis of the two groups produced similar results to separate analyses. Our urine collection was also not perfect. Thus, not all specimens were collected at the same time of the menstrual cycle. Also, we were unable to obtain one of the six urine samples from five women. However, subanalysis that only included urine samples from mid-luteal collections did not change the observed findings. Another shortcoming, like the ELISA method, is the inability of the GCMS method to measure 4-OH E_1, as direct radioimmunoassay kits are only available for C2- and C16α-OH E_1. Given the possible genotoxic effect of 4-OH E_1, a future analysis should expand the use of the LCMS method (32) that has the capability of assessing as many as 15 E_1 or E_2 metabolites (33,34).

In conclusion, the observed favorable effect of 2 daily soy servings on urinary 2:16α-OH E_1 ratio in premenopausal women provides support for the protective role of soy against breast cancer. Recent availability of a novel method to measure additional estrogen metabolites may provide further insight into soy's influence on modifying estrogen metabolism, as well as the impact of being an equol producer and the possible differences by menopausal status. On the other hand, it may be difficult to detect strong effects of soy foods in adulthood given the increasing evidence from epidemiologic studies protective effects are restricted to women in Asian countries who consume soy foods in early life (1,35).

Acknowledgments

This project was funded by grants from the National Cancer Institute (R01 CA 80843 and P30 CA 71789) and from the National Center for Research Resources (S10 RR 020890). Dr. S. Conroy was supported by grant R25 CA 90956. We thank all participants for their dedication and time to be part of this intervention.

References

1. Wu AH, Yu MC, Tseng CC, Pike MC. Epidemiology of soy exposures and breast cancer risk. Br J Cancer. 2008; 98:9–14. [PubMed: 18182974]
2. Xu X, Duncan AM, Merz BE, Kurzer MS. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. Cancer Epidemiol Biomarkers Prev. 1998; 7:1101–1108. [PubMed: 9865428]
3. Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis. 1998; 19:1–27. [PubMed: 9472688]
4. Lu LJ, Cree M, Josyula S, Nagamani M, Grady JJ, et al. Increased urinary excretion of 2-hydroxyestrone but not 16alpha- hydroxyestrone in premenopausal women during a soya diet containing isoflavones. Cancer Res. 2000; 60:1299–1305. [PubMed: 10728690]
5. Fuhrman BJ, Pfeiffer R, Xu X, Wu AH, Korde L, et al. Soy intake is associated with increased 2-hydroxylation and decreased 16alpha-hydroxylation of estrogens in Asian-American women. Cancer Epidemiol Biomarkers Prev. 2009; 18:2751–2760. [PubMed: 19789363]

6. Nebert DW. Elevated estrogen 16α-hydroxylase activity: is this a genotoxic or nongenotoxic biomarker in human breast cancer risk? J Natl Cancer Inst. 1993; 85:1888–1891. [PubMed: 8230275]
7. Bradlow HL, Davis DL, Lin G, Sepkovic D, Tiwari R. Effects of pesticides on the ratio of 16alpha/2-hydroxyestrone: a biologic marker of breast cancer risk. Environ Health Perspect. 1995; 103(Suppl 7):147–150. [PubMed: 8593862]
8. Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol. 1996; 36:203–232. [PubMed: 8725388]
9. Nettleton JA, Greany KA, Thomas W, Wangen KE, Adlercreutz H, et al. The effect of soy consumption on the urinary 2:16-hydroxyestrone ratio in postmenopausal women depends on equol production status but is not influenced by probiotic consumption. J Nutr. 2005; 135:603–608. [PubMed: 15735101]
10. Kabat GC, Chang CJ, Sparano JA, Sepkovie DW, Hu XP, et al. Urinary estrogen metabolites and breast cancer: a case-control study. Cancer Epidemiol Biomarkers Prev. 1997; 6:505–509. [PubMed: 9232337]
11. Ursin G, London S, Stanczyk FZ, Gentzschein E, Paganini-Hill A, et al. Urinary 2-hydroxyestrone/16alpha-hydroxyestrone ratio and risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 1999; 91:1067–1072. [PubMed: 10379970]
12. Fowke JH, Qi D, Bradlow HL, Shu XO, Gao YT, et al. Urinary estrogen metabolites and breast cancer: differential pattern of risk found with pre- versus post-treatment collection. Steroids. 2003; 68:65–72. [PubMed: 12475724]
13. Meilahn EN, De SB, Allen DS, Fentiman I, Bradlow HL, et al. Do urinary oestrogen metabolites predict breast cancer? Guernsey III cohort follow-up. Br J Cancer. 1998; 78:1250–1255. [PubMed: 9820189]
14. Muti P, Bradlow HL, Micheli A, Krogh V, Freudenheim JL, et al. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women. Epidemiology. 2000; 11:635–640. [PubMed: 11055622]
15. Falk RT, Fears TR, Xu X, Hoover RN, Pike MC, et al. Urinary estrogen metabolites and their ratio among Asian American women. Cancer Epidemiol Biomarkers Prev. 2005; 14:221–226. [PubMed: 15668498]
16. Ziegler RG, Hoover RN, Pike MC, Hildesheim A, Nomura MY, et al. Migration Patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst. 1993; 85:1819–1827. [PubMed: 8230262]
17. Maskarenic G, Morimoto Y, Conroy SM, Pagano IS, Franke AA. No changes in nipple aspirate fluid volume as the result of a randomized, 6-month soy food intervention among premenopausal women. J Nutr. 2011 [In Press].
18. Stram DO, Hankin JH, Wilkens LR, Henderson B, Kolonel LN. Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. Am J Epidemiol. 2000; 151:358–370. [PubMed: 10695594]
19. Franke AA, Halm BM, Kakazu K, Li X, Custer LJ. Phytoestrogenic isoflavonoids in epidemiologic and clinical research. Drug Test Anal. 2009; 1:14–21. [PubMed: 20355154]
20. Franke AA, Halm BM, Custer LJ, Tatsumura Y, Hebshi S. Isoflavones in breastfed infants after mothers consume soy. Am J Clin Nutr. 2006; 84:406–413. [PubMed: 16895891]
21. Maskarinec G, Hebshi S, Custer L, Franke AA. The relation of soy intake and isoflavone levels in nipple aspirate fluid. Eur J Cancer Prev. 2008; 17:67–70. [PubMed: 18090913]
22. Shackleton CH. Profiling steroid hormones and urinary steroids. J Chromatogr. 1986; 379:91–156. [PubMed: 3525596]
23. Xu X, Duncan AM, Wangen KE, Kurzer MS. Soy consumption alters endogenous estrogen metabolism in postmenopausal women. Cancer Epidemiol Biomarkers Prev. 2000; 9:781–786. [PubMed: 10952094]
24. Martini MC, Dancisak BB, Haggans CJ, Thomas W, Slavin JL. Effects of soy intake on sex hormone metabolism in premenopausal women. Nutr Cancer. 1999; 34:133–139. [PubMed: 10578479]

25. Brown BD, Thomas W, Hutchins A, Martini MC, Slavin JL. Types of dietary fat and soy minimally affect hormones and biomarkers associated with breast cancer risk in premenopausal women. Nutr Cancer. 2002; 43:22–30. [PubMed: 12467131]
26. Lampe JW. Is equol the key to the efficacy of soy foods? Am J Clin Nutr. 2009; 89:1664S–1667S. [PubMed: 19357217]
27. Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr. 2002; 132:3577–3584. [PubMed: 12468591]
28. Franke AA, Lai JF, Halm BM, Pagano I, Kono N, et al. Equol production changes over time in postmenopausal women. J Nutr Biochem. 2011 In Press.
29. Franke AA, Lai JF, Morimoto Y, Maskarenic G. Equol production over time in pre-menopausal women. Br J Nutr. 2011 In Press.
30. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. Nutr Cancer. 2006; 55:1–12. [PubMed: 16965235]
31. Faupel-Badger JM, Fuhrman BJ, Xu X, Falk RT, Keefer LK, et al. Comparison of liquid chromatography-tandem mass spectrometry, RIA, and ELISA methods for measurement of urinary estrogens. Cancer Epidemiol Biomarkers Prev. 2010; 19:292–300. [PubMed: 20056650]
32. Nelson RE, Grebe SK, OKane DJ, Singh RJ. Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma. Clin Chem. 2004; 50:373–384. [PubMed: 14656902]
33. Xu X, Veenstra TD, Fox SD, Roman JM, Issaq HJ, et al. Measuring fifteen endogenous estrogens simultaneously in human urine by high-performance liquid chromatography-mass spectrometry. Anal Chem. 2005; 77:6646–6654. [PubMed: 16223252]
34. Falk RT, Xu X, Keefer L, Veenstra TD, Ziegler RG. A liquid chromatography-mass spectrometry method for the simultaneous measurement of 15 urinary estrogens and estrogen metabolites: assay reproducibility and interindividual variability. Cancer Epidemiol Biomarkers Prev. 2008; 17:3411–3418. [PubMed: 19064556]
35. Messina M, Hilakivi-Clarke L. Early intake appears to be the key to the proposed protective effects of soy intake against breast cancer. Nutr Cancer. 2009; 61:792–798. [PubMed: 20155618]

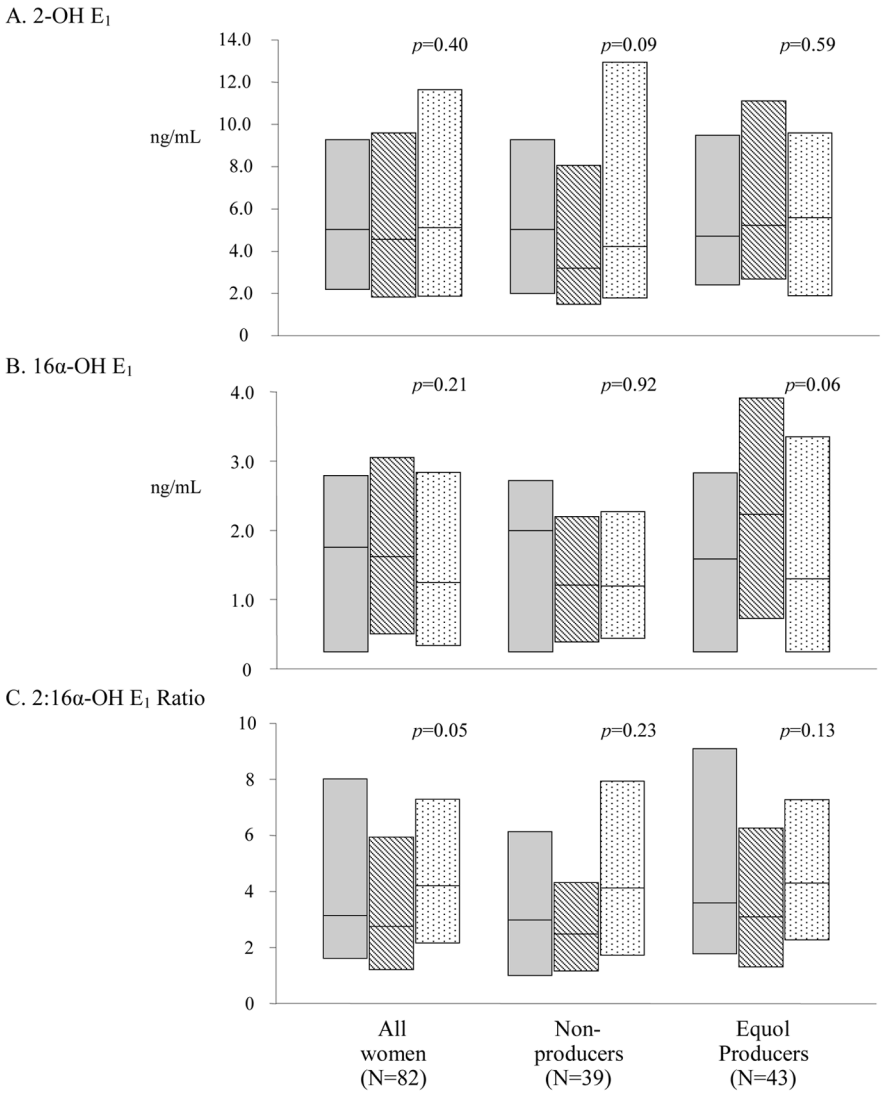

Figure 1.

Median and upper and lower quartiles of urinary 2-OH estrone (E_1) and 16α-OH E_1 excretions by equol producer status at baseline (gray) and after low-soy (stripe) and after high-Soy (dot) diets; Equol producer status is defined as urinary daidzein excretion ≥2 nmol/mg and urinary equol to daidzein ratio ≥0.018; P value for high-soy diet, adjusted for time, based on log-transformed data; One equol-producer had missing data at baseline (N=42); two non-producers at the high-soy diet (N=37); and another one equol-producer (N=42) and one non-producer (N=38) at the low-soy diet.

Table 1

Baseline characteristics of the study participants by randomization group[1]

Characteristic	All	Group A[2]	Group B[2]	*P* value
N	82	40	42	
Ethnicity				
White	42 (51%)	20 (50%)	22 (52%)	0.98
Asian	22 (27%)	11 (23%)	11 (22%)	
Other	18 (22%)	9 (27%)	9 (26%)	
Age at screening, *y*	39.2±6.1	41.3±5.6	37.3±6.0	<0.01
BMI, kg/m^2	25.8±5.6	25.8±5.2	25.9±6.0	0.91
Age at menarche, *y*	12.4±1.4	12.4±1.2	12.4±1.5	0.99
Number of children	1.5±1.3	1.6±1.1	1.4±1.5	0.40
Age at first live birth[3], *y*	27.8±6.7	27.7±7.1	28.0±6.4	0.87
Breastfeeding duration[3], *mo*	22.2±29.8	19.7±24.7	25.1±34.9	0.50
Equol producer status[4]	43 (52%)	23 (58%)	20 (48%)	0.37
Usual soy food intake, *g/day*[5]	6±9	5±8	7±10	0.44
Isoflavone intake, *mg/d*	21.2±39.7	16.3±38.8	25.8±40.5	0.28
Urinary isoflavones at randomization, *pg/mL*	499±925	728±1133	281±607	0.03
Urinary estrogen excretion, *ng/mL*[3]				
Estrone (E_1)	9.4±8.7	8.8±6.0	10.0±10.7	0.54
2-OH E_1	7.6±9.4	7.8±7.3	7.4±11.1	0.85
16α-OH E_1	2.1±2.3	2.2±1.5	2.11±2.8	0.93
2:16α-OH E_1 ratio	10.7±22.3	9.4±22.7	12.0±22.2	0.61
Estradiol (E_2)	3.3±3.0	3.1±2.4	3.5±3.5	0.51
Estriol (E_3)	10.8±9.8	10.3±7.1	11.2±11.8	0.68

[1] Data are n (%) or mean ± SD

[2] Group A: high-soy diet first, then low-soy diet; Group B: low-soy diet first, then high-soy diet.

[3] N = 59 for parous women; breast-feeding duration is missing for one woman in Group B and urinary estrogen excretion for one woman in Group A.

[4] Equol producer status is defined as urinary daidzein excretion ≥2 nmol/mg and urinary equol to daidzein ratio ≥0.018.

[5] Estimated based on a 1-year food frequency questionnaire completed at baseline; missing data for one women in Group B (N=41).

Table 2

Soy food intake, body weight, and urinary isoflavones and estrogens during the soy trial (baseline and at the end of the low- and high-soy diet) by equol producer status[1]

Equol producer status	Study phase	Soy food intake, *g/day*[2]	Body weight, *kg*	Urinary isoflavone excretion, *pg/mL*	Urinary estrogen excretion[3], ng/mL					
					Estrone (E_1)	2-OH E_1	16α-OH E_1	2:16α OH-E_1 ratio	Estradiol (E_2)	Estriol (E_3)
All women (N=82)	Baseline	6±9	67.1±14.5	499±925	6.4 (4.0–13.2)	5.0 (2.2–9.3)	1.8 (0.3–2.8)	3.2 (1.6–8.0)	2.4 (1.4–4.2)	7.6 (3.9–15.2)
	Low-soy	6±15	68.0±16.5	522±1237	7.3 (3.9–13.0)	4.6 (1.8–9.6)	1.6 (0.5–3.1)	2.8 (1.2–6.0)	2.3 (1.4–4.3)	7.8 (3.8–13.6)
	High-soy	58±37[4]	67.9±15.8	5624±6888[4]	7.6 (3.7–14.4)	5.1 (1.9–11.6)	1.3 (0.3–2.8)	4.2 (2.2–7.3)	2.4 (1.2–4.4)	7.1 (3.3–14.4)
Non-producers (N=39)	Baseline	5±6	67.3±17.3	463±981	8.0 (4.0–13.6)	5.0 (2.0–9.3)	2.0 (0.3–2.7)	3.0 (1.0–6.1)	2.5 (1.5–4.6)	8.5 (3.7–15.8)
	Low-soy	4±6	69.3±20.7	312±765	6.0 (3.5–13.0)	3.2 (1.5–8.1)	1.2 (0.4–2.2)	2.5 (1.2–4.3)	2.1 (1.4–4.5)	4.8 (3.0–10.4)
	High-soy	58±38[4]	68.7±19.7	4278±5798[4]	7.8 (3.6–15.1)	4.2 (1.8–13.0)	1.2 (0.4–2.3)	4.1 (1.7–7.9)	3.0 (1.3–4.7)	6.5 (3.5–12.3)
Equol producers (N=43)	Baseline	7±11	67.0±11.8	532±881	6.2 (4.5–13.2)	4.7 (2.4–9.5)	1.6 (0.3–2.8)	3.6 (1.8–9.1)	2.3 (1.3–3.6)	7.1 (4.4–14.8)
	Low-soy	8±20	66.8±11.5	716±1537	8.0 (4.2–13.0)	5.2 (2.7–11.1)	2.2 (0.7–3.9)	3.1 (1.3–6.3)	2.6 (1.3–4.1)	9.8 (4.4–16.9)
	High-soy	58±35[4]	67.2±11.4	6844±7608[4]	7.1 (3.8–13.6)	5.6 (1.9–9.6)	1.3 (0.3–3.3)	4.3 (2.3–7.3)	2.1 (1.1–4.0)	7.2 (3.0–20.2)[4]

[1] Mean±SD except for urinary estrogen excretion shown in median (and interquartile range) due to non-normal distributions.

[2] According to 1-year food frequency questionnaire at baseline, and mean of 3 recalls from each diet period.

[3] One equol-producer had missing data at baseline (N=42); two non-producers at the high-soy diet (N=37); and another one equol-producer (N=42) and one non-producer (N=38) at the low-soy diet.

[4] P value significant for high-soy diet adjusted for time (and based on log-transformed data for urinary estrogens); soy food intake: $p<0.0001$ for all; urinary isoflavone excretion: $p<0.0001$ for all; E3: $p=0.03$.